Original Research Article

# Maternal chitosan oligosaccharide intervention optimizes the production performance and health status of gilts and their offspring

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ABSTRACT

Chitosan oligosaccharides (COS) are the hydrolyzed product of chitosan and have multifunctional health benefits. The objective of this study was to elucidate the effect of COS as a dietary supplement to gilts on their productivity and health and that of their litters. Gilts were randomly assigned to either a treatment ($n = 30$) or control group ($n = 30$). The treatment gilts were fed a standard dry sow ration supplemented with COS at 0.12 and 0.24 g/gilt per d during gestation and lactation, respectively, and the control group was fed the standard dry sow ration only. The body weight, reproductive performance, milk production and litter size for each gilt and body weight of corresponding litters were recorded. The serum immunoglobulins (IgA, IgG, IgM) and secretory immunoglobulin A (sIgA) concentrations of gilts and piglets and fecal sIgA concertation of gilts were measured by Enzyme-linked immunosorbent assay (ELISA). Our study showed that maternal COS supplementation 1) significantly increased gilt body weight in late pregnancy ($P < 0.05$), 2) significantly increased milk production of gilts at different stages (d 1, 3, 7 and 19) of lactation ($P < 0.05$), 3) significantly increased body weight gain of piglets at weaning ($P < 0.05$), 4) significantly increased the serum concentrations of IgM and sIgA in piglets, and sIgA in fecal sample of gilts ($P < 0.05$), and 5) tended to increase the pregnancy success rate ($P > 0.05$) in the treatment group compared to the control group. These results suggest that maternal COS intervention in gilts can improve gilt milk production, piglet pre-weaning growth and immunity parameters in both gilts and piglets.

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## 1. Introduction

Feed additives are routinely used to stabilize health and metabolic status and promote the performance indices of intensively produced farm animals. Chitosan oligosaccharides (COS), which are widely found in nature and are a component of the exoskeleton of many arthropods—including shrimp, crabs and insects (Singla and Chawla, 2001)—are a relatively new and novel feed ingredient that

have potential as a feed additive for animals. COS has a low molecular weight, good solubility and low viscosity (Kim and Rajapakse, 2005) and is easily absorbed via the intestinal epithelium (Fang et al. 2015). Therefore, it has been used in many fields including food technology, human nutrition (Patel and Goyal, 2010) and medication (Zou et al. 2016).

COS has multiple biological functions including anti-microbial (Holappa et al., 2006), anti-inflammatory (Ma et al. 2011), anti-oxidative (Yen et al. 2008), anti-tumor (Shen et al. 2009), immunostimulatory (Zaharoff et al. 2007) and hypocholesterolemic (Liu et al. 2008) properties. It can optimize the growth and development of weaned piglets (Liu et al. 2008). Tang et al. (2005) showed that a diet containing COS increased plasma growth hormone (GH) and insulin-like growth factor-1 (IGF-1) concentrations in early-weaned pigs which may improve growth and feed conversion efficiency. COS was reported to provide an additional advantage to intestinal mucosal immunity by reducing intestinal inflammation

evident by decreased expression of Toll-like receptor 4 (TLR4) mRNA in the jejunal mucosa and also by increasing concentration of sIgA (Xiao et al. 2013). COS supplemented diet increased the total number of piglets born by 18.5% and the number of piglets born alive by 19.2% (Cheng et al. 2015). Significantly higher concentrations of different amino acids were reported in the milk of COS treated sows compared with the control group (Xie et al. 2015). In addition, COS aids in both nutrient digestibility and growth in broiler chickens and has also been shown to improve the tenderness of beef (Zhou et al. 2009).

Intrauterine growth restriction (IUGR) refers to a condition of the mammalian embryo/fetus, in which it does not reach its growth potential during gestation. The most common causes of IUGR include dysfunction of the placenta, endometrium, or uterus, disease, environmental stress and inadequate food intake (Wang et al., 2008). Moreover, a variety of physiological and production-imposed conditions are responsible for IUGR in livestock. This is most apparent with multifetal pregnancies in pigs, in which 15% to 25% of newborns have a birth weight of 1.1 kg compared with the average birth weight of 1.4 kg due to placental insufficiency (Wu et al., 2006; Wang et al., 2008). IUGR piglets show a decreased level of certain serum proteins, including immunoglobulin and annexin A1, which are important for the regulation of immune function. Furthermore, the development of feed additives that can help minimize the use of antibiotics within the pork industry while sustaining production efficiency will be important for the future sustainability of pig production globally. Although COS has multiple health benefits for both humans and animals, its role during pregnancy and lactation of gilts remains unexplored. Therefore, we hypothesized that maternal COS dietary intervention to gilts during pregnancy and lactation has a positive impact on performance and immune function of both gilts and their piglets.

## 2. Materials and methods

This study was approved by the Animal Care and Ethics Committee, Charles Sturt University, Wagga Wagga, NSW 2678, Australia.

### 2.1. Animal management and feeding

A total of 60 gilts (*Sus scrofa*), which were approximately 220 d of age and had an average body weight of 152 ± 4.6 kg, were randomly selected from 3 breeding lines (*Sus scrofa domesticus*). Breed lines consisted of pure-bred Large Whites, pure bred Landrace and pure bred Durocs. All gilts were housed in commercial gestation and farrowing facilities at the Pig Improvement Company, Grong Grong, New South Wales (NSW), Australia. Gilts were mated via artificial insemination at their first standing oestrus after pubertal oestrus was observed, and were randomly allocated to 2 groups, stratified according to the same genetic background by including a similar number of gilts from each breed line matched for body weight in the treatment and control groups. The treatment group ($n = 30$) was offered a standard dry sow and lactation ration with dietary supplementation of COS (Dalian GlycoBio Co. Ltd) during gestation and lactation, respectively. The control group ($n = 30$) was maintained on the same standard dry sow and lactation rations without COS supplementation. Therefore, the gilts were used as the experimental unit in this study.

The number of confirmed pregnant gilts in the COS and control groups was 20 and 19, respectively. However, 2 gilts from the COS group aborted during gestational week 5 and thus the total number of gilts entering the farrowing shed was 18 for the COS group and 19 for the control group. The non-pregnant gilts were used only to determine the pregnancy rate after the second mating by artificial

insemination. Three days before the estimated date of farrowing (gestation length 114 ± 1 d), the gilts were moved from the gestation shed to the farrowing shed and randomly allocated to an individual crate for farrowing. The farrowing sheds were maintained at a constant temperature of 22 °C. In commercial farming systems, fostering piglets from one gilt/sow to another is an established husbandry practice. In our study, the piglets were fostered to other gilts of the same treatment group to ensure 11 piglets were included in each litter. Any piglets cross-fostered from the COS group to control group or reverse were not used in the data analysis.

### 2.2. COS supplementation

COS was mixed with the commercial gestation and lactation feed at a dose rate of 50 g/t (50 mg/kg). Gilts allocated to the COS treatment were fed 2.4 kg/d (0.12 g of COS/d) from mating until the end of gestation. From d 1 after farrowing until d 19 of lactation, the amount of feed offered was increased to 4.8 kg/d (0.24 g of COS/d), which was fed across 2 feeding periods at 07:00 and 15:00 to ensure continuous access to feed. The control gilts were fed a commercial gestation and lactation ration at the same feeding level and time as for the COS treatment group. The nutritional composition of the gestation and lactation diet is provided in Appendix Table 1. All gilts were provided with water ad libitum throughout the experimental period.

### 2.3. Monitoring of pregnancy rate of gilts

At d 28 of gestation, the success of the pregnancy of all gilts was tested by ultrasound (Draminski ANIMAL profi, Poland). The number of gilts confirmed pregnant in the COS and control groups were 20 and 19, respectively. The non-pregnant gilts in COS group ($n = 10$) and in the control group ($n = 11$) were included in the experiment only to generate the second pregnancy rate after the second mating when they next displayed oestrus. The second pregnancy rate was calculated according to the success of this subsequent mating. The third pregnancy rate was determined in weaned sows ($n = 18$ for COS and $n = 19$ for control) which were mated 134 to 139 d after commencement of their initial COS supplementation.

### 2.4. Monitoring of other reproductive performance of gilts

Reproductive performance of each gilt was monitored by assessing the total number of piglets born, those born alive, those born dead and those categorized as IUGR piglets. Piglets with body weight less than 1,000 g were considered as IUGR piglets.

### 2.5. Measurement of body weight of gilts and piglets

Before insemination, the baseline (d 1) individual body weight of gilts was recorded to ensure that both the COS and control group gilts started treatment with a similar body weight. To examine the health benefits of COS intervention, all gilts from both groups were weighed at 5 time points (d 30, 60, 74, 90 and 104) during gestation. In the first month after insemination, the gilts were not weighed until the d 30 pregnancy check, in order to minimize pregnancy disturbance during the period of embryo attachment. The individual body weight of lactating gilts on postnatal d 19 was also recorded to compare with the baseline body weight at d 1. Individual weights of gilts were measured using a VD-1000Vet Deck veterinary scale (Slater Brecknell, UK) with 500 kg × 0.1 kg capacity.

Piglets were individually weighed within 24 h of birth and before ear tagging and tail docking occurred. Individual weight was

also recorded on d 19 before weaning. Piglets were weighed using an ElectroSamson-Digital hand held scale (Slater Brecknell, USA) with maximum capacity of 25 kg × 0.02 kg.

## 2.6. Measurement of milk production of gilts

Milk production per suckling episode was assessed through piglet live weight changes over a suckling bout (weigh-suckle-weigh) and was determined on postnatal d 1, 3, 7 and 19. Milk production was measured by isolating the piglets from the sow using a plastic board for 60 min after which the piglets were then weighed individually and the plastic board was removed allowing the piglets to suckle. Piglets were allocated 15 min to suckle or until suckling was completed. The piglets were weighed individually and the weight gain of the litter was used as an indication of gilt milk production per suckling episode. This procedure was repeated at least 3 times in the absence of any defecation or urination by piglets while suckling and weighing in order to determine the average milk production per suckling bout on that particular day of lactation. If there was any defecation or urination, the process was repeated at each time point to allow for accurate determination of gilt milk production.

## 2.7. Immunoglobulin assays

On postnatal d 19, blood samples were collected from all gilts and from one piglet, selected randomly, from each litter. Three millilitres and 2 mL of blood were collected from the jugular vein of gilts and piglets, respectively, and placed into 4-mL serum clot activator tubes (Greiner Bio-one, Australia). Separation of serum was conducted by centrifuging the blood sample at 3,000 × $g$ for 10 min at 4 °C. Finally, the serum samples were stored at −80 °C for further analysis. Only those serum samples which were devoid of hemolysis were used for immunoglobulin assay by Enzyme-linked immunosorbent assay (ELISA).

Fecal samples were collected from the gilts between 105 and 111 d of gestation and stored at −80 °C. The processing of fecal samples for secretory immunoglobulin A (sIgA) analysis was carried out following the instructions of the ELISA Kit (My Biosource, USA, Catalog no CSB-E12063p). Briefly, 100 mg of fecal sample was mixed with 5 mL of wash buffer on a vortex mixer at room temperature until the mixture was homogenous. One milliliter of the mixture was transferred into an Eppendorf tube and was centrifuged at 10,000 × $g$ for 10 min. The supernatant was diluted at 1:250 with wash buffer (4 μL + 996 μL wash buffer) with 100 μL of the diluent being used per well.

A pig immunoglobulin M (IgM) ELISA kit (Life Diagnostics, Inc. USA, Catalog no. 5015-9), immunoglobulin G (IgG) and immunoglobulin A (IgA) ELISA kits (Bethyl Laboratories, Inc. USA, Catalog no. E101-104 and Catalog no. E100-102) were used to determine the serum concentrations of IgM, IgG, and IgA, respectively. A pig sIgA kit was used to detect sIgA concentration in gilt and piglet serum as well as in the gilt fecal samples. All samples were analysed in duplicate and the intra- and inter-assay coefficients of variation were less than 5%. An automated plate washer (ELx50 Washer, BioTek) was used to wash the plates. A microplate spectrophotometer (SpectraMax, Bio-strategy) was used to measure the optical density of serum samples at a wave length of 450 nm.

## 2.8. Statistical analysis

Linear mixed models were used to evaluate the effects of COS on pregnancy success, milk production, litter size, birth weight of piglets, also on body weight gain and immunity of gilts and piglets. To accommodate the experimental design, gilt nested within breed line was used as the random effect and treatment group was used as a fixed effect in the case of gilt pregnancy success, gilt and piglet immunity, litter size and birth weight of piglet. Whereas, in the case of gilt body weight gain, milk production and piglet body weight gain, gilt nested within breed line was used as the random effect and treatment group, days and their interactions were used as fixed effects. Residual plots were used to ensure that the model assumptions were met for all models. The difference in the incidence of IUGR between COS and control groups was analysed using linear mixed models (binomial generalised mixed models). Comparisons of pregnancy success rate between COS and control group were carried out using one-way ANOVA and Fisher's LSD. The differences between the COS treatment and control groups were considered to be significant when $P < 0.05$. The analysis was conducted using R statistical software (R Core Team, 2015). The R packages nlme and lme4 were used. Additionally, SPSS software for windows (IBM SPSS statistics 20) was used to check the $P$-value.

## 3. Results

### 3.1. Effects of COS on body weight of gilts during gestation

The mean live body weight of gilts on d 1, 30, 60, 74, 90 and 104 of gestation is illustrated in Fig. 1A. Chitosan oligosaccharide supplementation had no significant effects on gilt gestational body weight gain at the first 4 stages of gestation (d 1 to 90) ($P > 0.05$), however there was a significant increase in body weight gain in the COS group compared to the control group ($P = 0.02$) at the last stage (d 91 to 104 of gestation).

No significant difference in mean body weight gain per day between the 2 groups ($P > 0.05$) was found at 5 stages of gestation (Fig. 1B). However, the gilts from the 2 groups gained more than 0.5 kg/d, except for stage 3, in which the COS treatment gilts only gained 0.361 kg/d, while the control gilts gained 0.43 kg/d. Although the mean body weight gain per day of the gilts fed COS tended to be higher than that of the control gilts at the first 2 stages and the last 2 weeks of gestation (Stage 5), the differences between 2 groups did not reach a significant level ($P > 0.05$).

### 3.2. Effects of COS on pregnancy success of gilts

All gilts were tested for pregnancy on d 28 after insemination. The results of the pregnancy test are summarized in Table 1. The first pregnancy rate of the COS group was slightly higher than that of the control group. Twenty out of 30 (66.67%) gilts in the COS treatment were found to be pregnant. Meanwhile, 19 out of 30 gilts (63.33%) were found to be pregnant in the control group. The difference between the groups was not significant ($P > 0.05$). A total of 21 non-pregnant gilts (control $n = 11$, COS $n = 10$) was artificially inseminated again at their first observed standing oestrus after confirmed negative pregnancy test. All gilts from the COS group that failed to become pregnant the first time, became pregnant. Meanwhile, less than 55% of the control gilts that failed to become pregnant at their first-time insemination became pregnant after the second insemination ($P < 0.05$, Table 1). These results indicated that the pregnancy rate, as a result of the subsequent mating of gilts fed COS was significantly higher than that of the control gilts ($P = 0.013$, Table 1). When the weaned first parity sows were re-mated, a ~15% higher pregnancy rate was observed for the COS group (89.89%) compared with the control group (73.68%) ($P > 0.05$, Table 1).
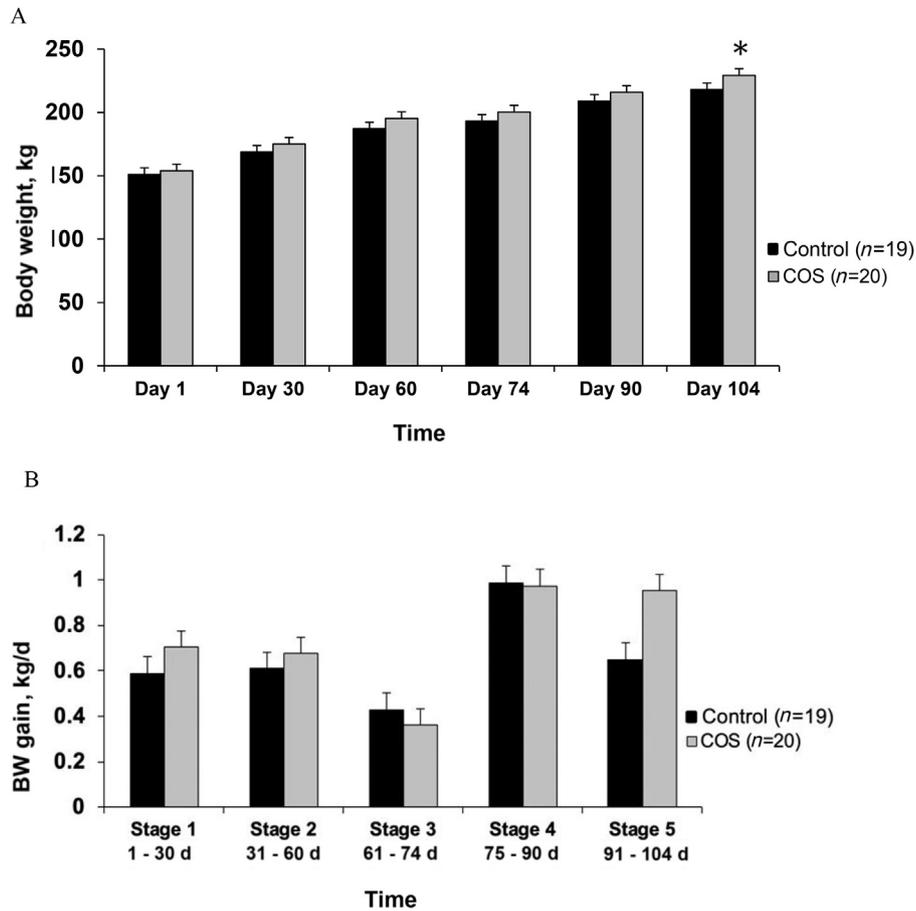
**Fig. 1.** Comparison of body weight and body weight gain per day between COS treatment and control gilts during gestation. (A) The mean (±SEM) of body weight (kg) of the COS treatment ($n = 20$, from d 1 to 30, and $n = 18$ from d 60 to 104) and control gilts ($n = 19$) during gestation. (B) The mean (±SEM) of body weight gain per day (kg/d) of the gilts from COS treatment and control during 5 stages of gestation. *, P-value < 0.05 between the 2 groups. COS = chitosan oligosaccharides.

**Table 1**
The incidence of pregnancy in the COS supplemented and control groups.

| Item | COS group | | | Control group | | | *P*-value |
|---|---|---|---|---|---|---|---|
| | Total mated | Total Pregnant | Pregnancy rate, % | Total mated | Total pregnant | Pregnancy rate, % | |
| 1st mating (gilts on heat) | 30 | 20 | 66.7 | 30 | 19 | 63.3 | 0.791 |
| 2nd mating (gilts returned to heat) | 10 | 10 | 100 | 11 | 6 | 54.55 | 0.013 |
| 3rd mating (weaned sows on heat) | 18 | 16 | 88.89 | 19 | 14 | 73.68 | 0.25 |

COS = chitosan oligosaccharides.

### 3.3. Effects of COS on birth performance

Maternal COS supplementation did not result in a significant difference in litter size or overall individual birth weight compared to the control group. No significant difference was observed in the number of piglets born alive, of IUGR piglets and total dead piglets between the COS supplemented and control groups ($P > 0.05$, Table 2).

### 3.4. Effects of COS on milk production

Colostrum, transition and mature milk production was measured at 4 time points (d 1, 3, 7 and 19) of lactation (Fig. 2A). The mean milk produced by the gilts receiving the COS treatment and control group was 350.23 ± 52.67 g and 272.03 ± 50.83 g on d 1, 296.89 ± 51.172 g and 219.97 ± 52.67 g on d 3, 299.46 ± 52.67 g and 255.33 ± 49.17 g on d 7, and 622.20 ± 49.17 g and 408.13 ± 49.17 g

on d 19, respectively. COS supplemented gilts produced approximately 29%, approximately 34%, approximately 17% and approximately 53% more milk than the control gilts over the 4 time points studied ($P = 0.02$, Fig. 2A).

**Table 2**
The effect of COS supplementation on litter related to reproductive performance of gilts.

| Item | COS group ($n = 18$) | | Control group ($n = 19$) | | *P*-value |
|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | |
| Litter Size | 10.53 | 1.21 | 10.04 | 1.21 | 0.66 |
| Alive piglets | 163 | | 173 | | 0.95 |
| IUGR piglets | 16 | | 16 | | 0.88 |
| Mean newborn piglet body weight, kg | 1.33 | 0.33 | 1.35 | 0.38 | 0.98 |

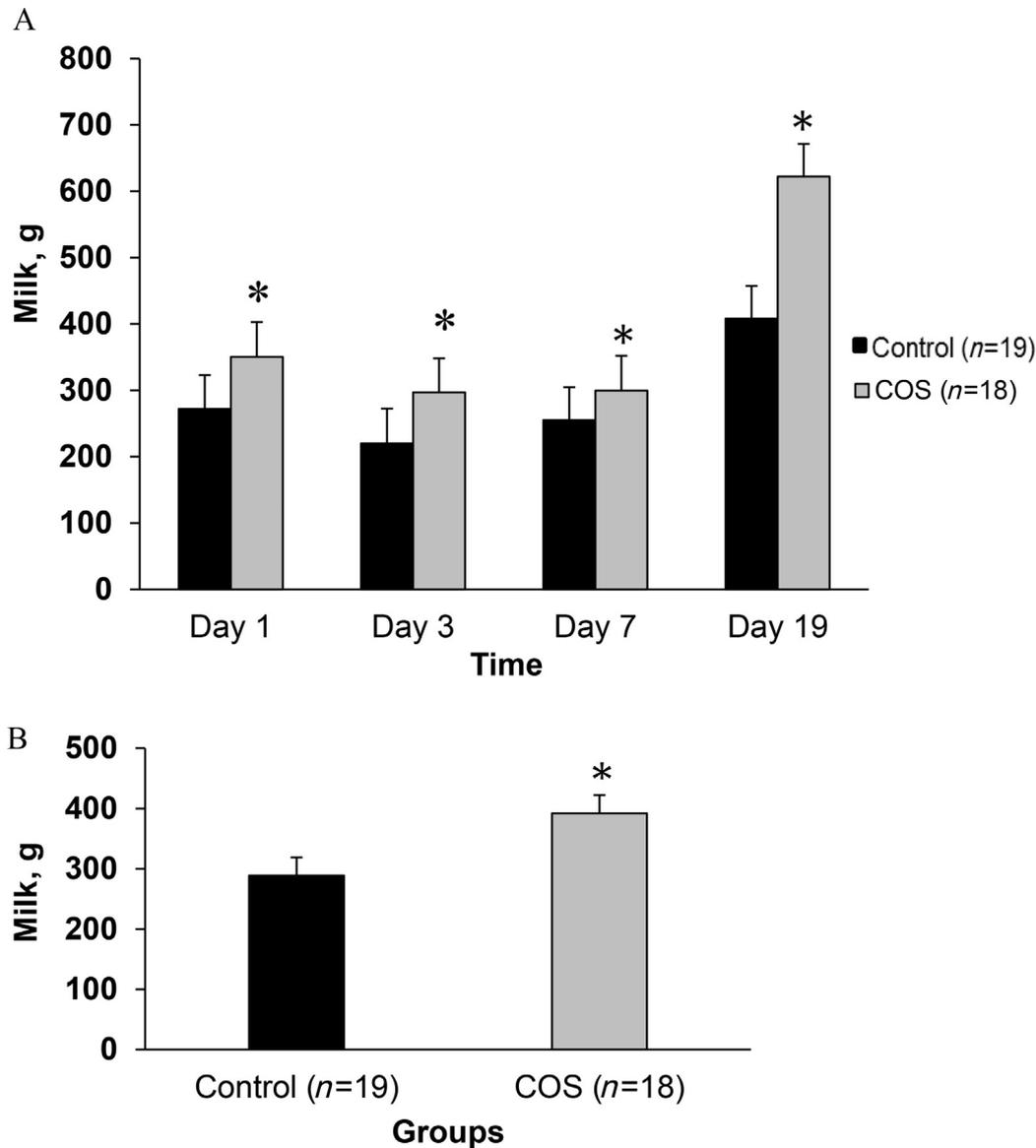COS = chitosan oligosaccharides; IUGR = intrauterine growth restriction.

**Fig. 2.** Comparison of milk production between COS treatment and control gilts during the course of lactation. (A) The mean (±SEM) of milk production (g) of the COS treatment and control gilts during 4 time points of lactation. *, *P* < 0.05. (B) Overall difference in milk production between COS treatment and control groups. *, *P* < 0.05. COS = chitosan oligosaccharides.

We also analysed milk production by combining all 4 time point results together. The results showed that the mean (±SEM) of overall milk production in the COS treatment group was 392.20 ± 30.01 g, which was significantly higher than for the control group (288.90 ± 29.66 g) (*P* < 0.05, Fig. 2B). The milk yield of COS treated gilts was increased by 35.8% relative to milk produced by the control gilts at the d 19 of lactation (*P* = 0.02, Fig. 2B).

### 3.5. Effect of COS on growth performance of piglets during lactation

Maternal COS intervention had beneficial influences on the growth performance of piglets during lactation by improving piglet body weight gain per d and body weight at weaning at d 19. The mean body weight gain of piglets (*n* = 97) from the supplemented COS group was 0.21 ± 0.01 kg per d, which was 16.48% higher than that for the control piglets (*n* = 99) (*P* = 0.005, Fig. 3A). The average body weight at weaning on d 19 in COS piglets (5.30 ± 0.20 kg) was

significantly higher than that of the control piglets (4.70 ± 0.20 kg) (*P* < 0.05, Fig. 3B).

### 3.6. Effects of COS on the immune response of gilts

Maternal COS intervention did not affect the expression levels of IgM, IgG, IgA and sIgA in blood serum samples of gilts at weaning (*P* > 0.05, Fig. 4A). However, the concentration of sIgA in faeces of the treated gilts was significantly increased on d 90 of gestation by 17.4% compared to the control group (*P* = 0.04, Fig. 4B).

### 3.7. Effects of COS on immune response of piglets

Maternal COS intervention not only optimized the growth performance of piglets, but also improved their immune status at weaning by increasing the concentrations of serum IgM and sIgA by 46.9% and 10%, respectively, compared with the control piglets (*P* < 0.05, Fig. 5). The difference in concentrations of IgA and IgG of
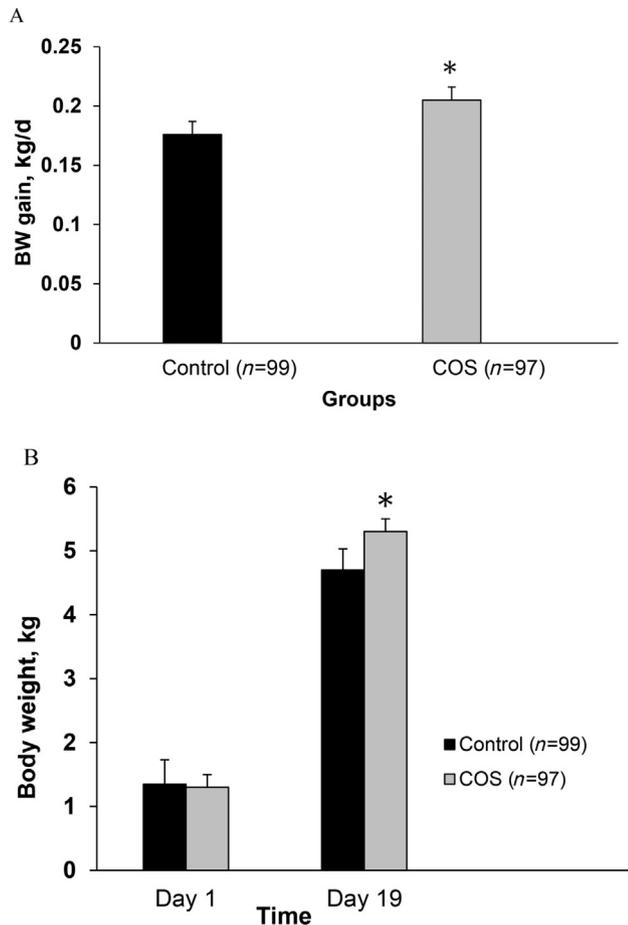
A



B



**Fig. 3.** Comparison of mean newborn body weight, body weight gain per day and postnatal d 19 weaning body weight of piglets between maternal COS treatment and control group. Only those piglets, which remained within the respective treatment ($n = 97$) or control ($n = 99$) group till weaning were weighed to record the weaning weight. (A) The mean body weight gains ($\pm$SEM) of piglets (kg/d) from the COS treatment and control groups during 19 d of lactation. *, $P < 0.05$. (B) The mean ($\pm$SEM) of live birth weight (d 1) and weaning weight (d 19) of piglets from the COS treatment and control groups. *, $P < 0.05$. COS = chitosan oligosaccharides.

A



B



**Fig. 4.** Expression levels of IgM, IgG, IgA, sIgA in serum and sIgA in faecal samples of the COS treatment and control gilts at 19 d after giving birth and 90 d of gestation, respectively. (A) The mean ($\pm$SEM) concentrations (mg/mL) of immune markers of IgM, IgG, IgA and sIgA in blood serum samples of the COS treatment and control gilts at weaning d 19 after giving birth. Samples without any hemolysis were used in this assay. (B) The mean ($\pm$SEM) of sIgA concentration (mg/mL) in faecal samples of the COS treatment and control gilts at d 90 of gestation. *, $P < 0.05$. COS = chitosan oligosaccharides.

piglets between the 2 groups was not statistically significant ($P > 0.05$, Fig. 5).

## 4. Discussion

Nutrition and functional feed additives play a significant role in enhancing reproductive efficiency in all animals and to our knowledge, this is the first time that the benefits of maternal COS intervention on reproductive performance of gilts has been demonstrated. The current study showed that maternal dietary supplementation with COS during gestation and lactation in gilts significantly improved: 1) the body weight gain of pregnant gilts in late pregnancy, 2) pregnancy rate of gilts in the second (COS 100% vs. control 54.55%, $P < 0.05$) and third (COS 88.89% vs. control 73.68%) insemination attempt, 3) gilt milk production throughout lactation, 4) pre-weaning piglet body weight gain, and 5) serum IgM and sIgA concentrations in piglets and fecal sIgA concentration in gilts.

Body condition during gestation is very important for pregnant pigs and their offspring (Xie et al. 2015). Dietary supplementation of COS increased body weight gain of gilts significantly at 104 d of gestation, but not earlier (d 1 to 90). Since the average birth weight
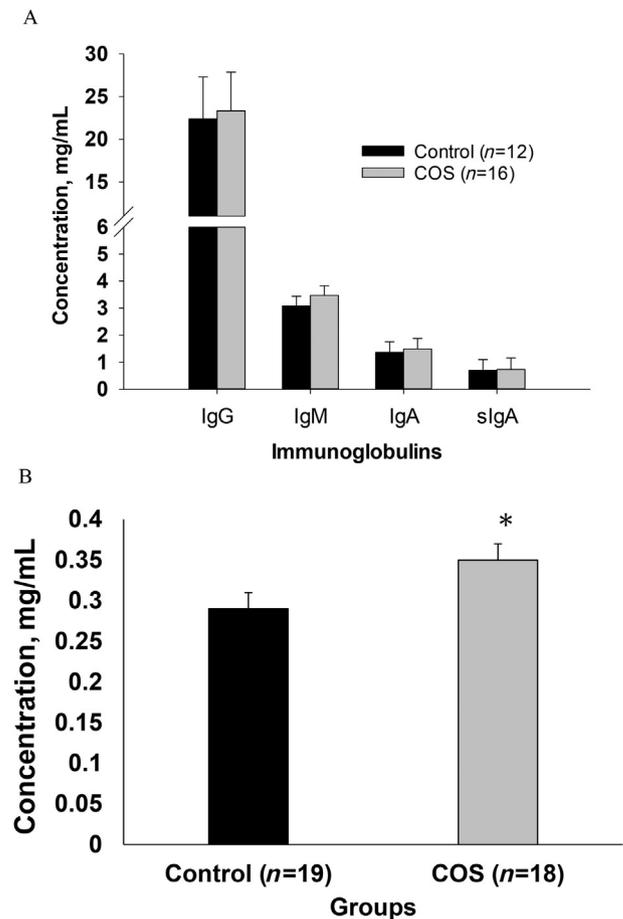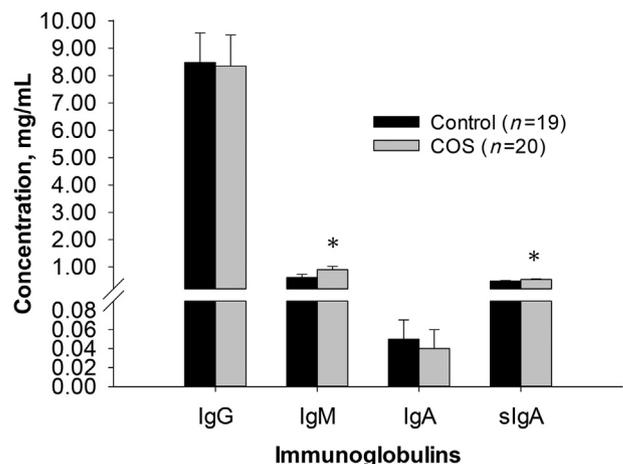


**Fig. 5.** The mean ($\pm$SEM) concentrations (mg/mL) of serum IgG, IgM, IgA and sIgA in piglets between maternal COS supplementation and control groups at weaning (postnatal d 19). *, $P < 0.05$. Samples without any hemolysis were used in this assay. COS = chitosan oligosaccharides.

of piglets in the treatment and control group was very similar, improved body condition of treatment gilts by COS intervention might be a possible reason behind this increased body weight.

The mechanism of action of COS on the physiological effects of milk production is not well understood. Nevertheless, the capacity of milk production by the mammary gland is dependent on the density of mammary epithelial cell and its biosynthetic capacity (Hurley, 2001; Oakes et al., 2006). COS is documented to have regenerative effects on nerve cell by stimulating proliferation of Schwann cells in a rat model (Wang et al., 2016). Thus, the increased milk production in COS treatment gilts might be the result of COS stimulating mammary epithelial cell proliferation and differentiation. Further study is required to confirm our proposed mechanism. Suckling intensity is a major determinant of both mammary development and milk yield of sows (Barber et al., 1955; Wilde and Peaker, 1990; Marshall et al., 2006; Riley et al., 2008). Therefore, increased litter size in COS group may provide a greater suckling intensity, which leads to greater oxytocin release and greater milk let-down in COS group.

In our study, the conception rate of gilts consuming COS was 66.67% in the first mating compared with the control group of 63.3% (Table 1). Although the COS group contained 10 non-pregnant gilts from the first mating, all of them became pregnant in their second mating, whereas 5 out of 11 gilts from the control group failed to conceive at the second mating (Table 1). This led to them being culled from the herd. Our current results demonstrated that COS significantly enhances the pregnancy rate of gilts, which has not been reported previously. However, the sample size is relatively small and a larger sample size study will be required to confirm our findings.

Maternal COS intervention increased the litter size by ~5%, but there was no statistically significant difference between the groups. Cheng et al. (2015) reported a 18.5% increase in the number of newborn piglets per litter compared with the control group using sows with parity 2 to 4. The current study was carried out on first parity (gilts) pigs, which might have not attained a mature body weight and uterine capacity compared with multiparous sows. Other reasons may be the difference in age of animal, nutrition in pregnancy and lactation performance between the 2 studies.

Maternal nutrition during gestation plays an essential role in the growth of foetuses and the survival of neonatal piglets (Hansen et al. 2012). Moreover, milk production is the key factor in determining the rate of piglet growth and development (Marshall et al., 2006). The significant increase in live weight gain of COS piglet corresponded to the increased milk availability associated with maternal COS supplementation. Increased colostrum intake might have enhanced growth velocity and immune status of piglets (Decaluwé et al. 2014). The increased supply of functionally important minor proteins, lipids, carbohydrates and non-nutritional products (Barber et al., 1955; Prunier et al., 2010) such as immunoglobulins, lactoferrin, macrophages and lysosomes in milk presumably provided increased immunoprotection and antimicrobial factors to the neonate (Bourne and Curtis, 1973). Part of the growth response to maternal COS supplementation may also be the result of the immunomodulatory effects of COS in significantly increasing the concentrations of serum IgM and sIgA, which led to a reduction in the incidence of early infectious diseases in the piglets.

COS has been found to increase the synthesis of protein, circulating IGF-I and GH concentrations in the blood of growing pigs (Tang et al., 2005), which may contribute to weight gain of COS

piglets to weaning. However, COS did not improve the birth weight of piglets; interestingly, birth weight of COS piglets was even lighter than the control piglets, which may have been due to the litter size of the COS group being 5% larger than the control group (Table 2).

Not all studies have found significant benefits in piglet growth performance in response to dietary COS supplements. Yan and Kim (2011) found that when they supplemented the diets of newly weaned piglets with COS at a dose of 3 mg/kg for 5 weeks, there was no growth response. Similar results were reported by Sun et al. (2009), with the direct supplementation of piglets post weaning. Thus, Xie et al. (2015) suggested that the maternal intervention with dietary COS was more effective on growth performance of suckling piglets than supplementing the diet of piglets directly with COS. Further studies should be conducted to compare the effects of COS on growth performance of piglets from gilt and sow litters, to elucidate this mechanism.

Some studies have investigated the impact of COS on the immune response of chickens (Huang et al. 2007), and weaned piglets (Li et al. 2013). However, there are very few reports on the effects of COS on circulating immunoglobulin concentrations in either sows or gilts during gestation and lactation. Although COS is known to stimulate the humoral immune response (Xia et al. 2011), our results showed that the concentrations of serum immunoglobulins (IgM, IgG, IgA, and sIgA) in gilts at weaning was not affected by dietary COS. The reason might be due to the coincidence of blood sampling with the commencement of lactation. In general the onset of lactation is immunosuppressive: for example Klobasa et al. (1985a,b) reported that the concentration of serum IgM declined over the course of lactation, reaching the lowest level in week 3, while the concentrations of IgG and IgA increased immediately post-partum (Klobasa et al. (1985a,b)). Serum immunoglobulin levels were tested on d 19 of lactation, immediately post-weaning to minimise stress on the gilts. Parity can also have an impact on immunocompetence. Klobasa et al. (1985a,b) reported that immunoglobulin concentrations of IgM, IgA, IgG increased with the increasing parity. For example, IgG was increased up to the fourth gestation, and IgM up to the third. In general, serum immunoglobulin was lower in first parity sows than in multiparous sows. To confirm these findings, more studies are required.

The 17.4% increase in the sIgA concentration in fecal samples resulting from COS treatment gilts (Fig. 4B, $P < 0.05$) may be important in protecting the intestinal epithelium from enteric toxins and pathogenic bacteria, fungi and viruses through immune exclusion (Mantis et al. 2011). Immune exclusion results from the ability of sIgA to block microbes and toxins from attaching to mucosal target epithelial cells, thus protecting against surface damage, colonisation and subsequent incursion (Mestecky et al. 1999). During gestation, none of the gilts suffered from any intestinal diseases. Thus, COS might play an important role in improving the gut immunity of gilts during gestation. The molecular mechanism responsible for COS induced sIgA expression in fecal sample needs further investigation. However, 2 gilts from the COS intervention group aborted at gestational week 5. It is possible that these gilts were more sensitive to the movement during the measurement of body weight during early stage gestation, which resulted in disturbance to the embryo attachment and survival.

The significant increases in IgM (46%) and sIgA (10%) in serum of piglets from gilts supplemented with COS piglets indicate that maternal COS intervention is associated with the increase in passive immune protection of offspring until their own immune system is developed (Rooke and Bland, 2002) (Fig. 5, $P < 0.05$).

Immunoglobulin M produced by B1 cells during the development of the foetus and neonate plays an important role in protecting against invasion of bacteria and viruses through the enhancement of IgG responses. However it may also be associated with the development of auto-reactive IgG and autoimmune disease (Boes, 2000), the impacts of which are unknown. The mechanism for the higher expression of serum IgM of piglets from gilts supplemented with COS at PND 19 requires further investigation as does its possible effects both during foetal and neonatal development. Secretory immunoglobulin A has a crucial function in the defence and homeostatic regulation of respiratory, intestinal and urogenital mucosal epithelia (Corthésy, 2013). The potential for dietary COS to stimulate functionally important immunoglobulin deserves further investigation. However, the immunomodulatory role of COS is selective since neither serum IgG nor IgA were improved by this dietary additive. The role of the maternal immune system is interesting here, as IgG is the only immunoglobulin that is transferred from mothers to the foetus via the placenta (Butler et al. 2009). In contrast, IgA is synthesized in the foetus and not transferred through the placenta. The role of the dam here is unclear since IgA levels in milk of gilts decrease during lactation (Butler et al. 2009) A number of studies have been conducted to investigate the impacts of COS on the immune status of animals. COS was found to enhance the immune status of broiler chickens by increasing the concentrations of IgG, IgA, and IgM in a 6-week experiment (Huang et al., 2007). The immune responses to dietary COS supplementation assessed in piglets at d 19 *post-partum* are potentially important for commercial pig production. However, the persistency of the response post-weaning needs to be investigated in establishing the commercial viability of the addition of COS to commercial gilt and sow gestational diets. Since the pig is an ideal animal model for translational nutrition studies in humans, it is likely that our findings here will have positive implications for human neonatal growth and immunity. Clearly this will be the subject of future investigations.

## 5. Conclusion

Chitosan oligosaccharides has the potential as a feed additive to improve pig production, milk production, and health state of pregnant and lactating gilts and their piglets. Further studies are required to explore the persistency of these responses in piglets post weaning and the molecular mechanisms associated with these responses.

## Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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## Appendix

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